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BANG'S DISEASE IN A NATURALLY INFECTED HERD[1]

F. M. HAYES[2] AND E. H. BARGER[3]

THE STUDIES to be reported herein were begun in 1922 in a herd of dairy cattle in which 19.0 per cent of the animals were infected with *Brucella abortus,* as indicated by the agglutination test of the blood serum. The particular objects were to determine what correlation exists between agglutinins in the blood and those in the milk, and what relation these agglutinins bear to the presence of the organisms in the milk and in the products of normally or prematurely terminated pregnancies.

Unfortunately the herd upon which these observations were made was not maintained solely for the purpose of these investigations, and the procedures carried out were necessarily limited in scope and in many cases were terminated by death or disposal of the animals or by their use for other purposes before conclusions could be drawn. However, certain of the animals have contributed significant data.

Previous to 1922 this herd was known to be infected with Bang's disease. Occasional abortions had occurred and agglutination tests on the blood had proved positive, but since there were no alarming symptoms of the disease, no particular effort was made to blood-test the animals systematically and regularly. The aborters were isolated until uterine discharges ceased and were then returned to the herd. From 1922, when 19.0 per cent were discovered to be reactors to the test, to 1926, more hygienic procedures were practiced with the aborters and the positive-reacting cows that calved normally, in an effort to control the spread of the disease. The disease remained stationary during 1923 and 1924 and increased in virulence in 1925 and 1926, and in these latter years reached one of the typical peaks that is characteristic of infected herds. Circum-

[1] Received for publication July 9, 1935.

[2] Professor of Veterinary Science and Veterinarian in the Experiment Station.

[3] Junior Veterinarian in the Experiment Station. Resigned May, 1928.

stantial evidence only, is available to account for the rise in the number of reactors and aborters in 1925 through the addition to the old herd in the spring of 1923 of 34 animals from another herd, 9 of which were reactors to the agglutination test.

METHODS

Blood and milk samples were collected on the same day at intervals of approximately 90 days, sometimes oftener. The blood and milk serums were then subjected to the agglutination test in the same dilutions with the same antigens. The greater part of the approximately 400 cc of the milk not used for the agglutination test was centrifuged and the sediment injected intraperitoneally into two guinea pigs. Whenever an abortion or a normal calving occurred, appropriate material was cultured for *Brucella abortus* on glycerine glucose gentian-violet agar, and two guinea pigs injected intraperitoneally with a salt-solution suspension of the material. Guinea pigs were killed at from five to six weeks, their blood secured for the agglutination test, and spleen, lungs, and liver cultured for *Br. abortus*.

Blood Agglutination Test.—Four dilutions of blood serum were made as a routine procedure by directly pipetting 0.020, 0.010, 0.005, and 0.002 cc of serum, to which 1.0 cc of antigen was added, thus making dilutions of 1–50, 1–100, 1–200, and 1–500. The antigen was prepared from 48 to 72-hour growths of several strains of bovine and porcine *Brucella abortus* by the addition of 0.5 per cent physiological sodium chloride and after thorough shaking by hand was filtered through coarse filter paper or a thin layer of cotton. Addition of carbol-saline was then made to secure a density of 3.4 Gates reading. No attempt was made to adjust the reaction to any particular pH concentration. This suspension was then stored in an ice box and used when the organisms proved to be dead. The serum-antigen tubes were incubated overnight, removed to room temperature, and readings made at 24 and 48 hours.

Collection and Treatment of the Milk Samples.—Approximately 400 cc of milk constituted the usual sample, which was collected in approximately equal amounts from all milking quarters. Ordinary market-milk pint bottles were covered with a layer of fine cheesecloth, the paper cap applied, and the whole top part of the bottle covered with medium heavy wrapping paper which was tied around the neck of the bottle with string. These bottles were then sterilized in the autoclave.

Ten to 20 cc of each sample was withdrawn from the bottle in a sterile manner to provide a sample for the whey-agglutination test. The rest of the milk was distributed in large sterile test tubes and centrifuged for

20 minutes, and the sediments mixed together and recentrifuged, so that the final sediment represented that from the entire sample. An equal quantity of the cream was always included with this final sediment for guinea-pig injection. Two guinea pigs were injected, intraperitoneally in most cases, with 2.0 cc of the mixed sediment and cream from each cow.

The portion of milk retained for the agglutination test was placed in a warming bath after the addition of a few drops of commercial rennet. When the whey had clearly separated, it was subjected to the test by using the same technique which was applied to the blood serum.

Collection and Treatment of Uterine Contents.—Whenever a fetus or a fresh and clean placenta was available, it was collected and brought to the laboratory for culturing and guinea-pig injection. In the absence of these, uterine swabs were taken. Cultures on glycerine glucose gentian-violet agar with a pH of 6.8–7.0 were made from the stomach contents, spleen, and liver of every available fetus, and from the placenta or uterine swabs. At least two guinea pigs were injected intraperitoneally with these materials.

The uterine swabs were collected by means of a stiff wire, at one end of which a piece of coarse cheesecloth was wrapped loosely and tied. The gauze end of this instrument was inserted into a large test tube, which was then sealed with a cotton plug. This was then covered with wrapping paper and the whole sterilized. In taking the uterine swab, the external genitals were thoroughly cleansed. The clean hand and arm then carried the unwrapped tube containing the gauze end of the wire to the cervical end of the vagina, where by a little manipulation the glass tube was removed from the gauze swab. The swab was then pushed into the uterus and its walls wiped until the gauze was assumed to be saturated, when it was withdrawn to the vagina, again inserted into the glass tube, brought to the outside, and recovered with the sterile paper wrapper, without having been exposed to contamination from the exterior. In the laboratory, from 10 to 15 cc of sterile physiological sodium chloride was added to sterile tubes and a suspension prepared from the swabs. The contents remaining from the washed gauze swabs were then centrifuged and the sediment cultured and injected into guinea pigs in the same manner as for the other materials already described.

CORRELATION BETWEEN AGGLUTININS AND UDDER INFECTION

Blood Agglutinins.—Simultaneous blood agglutination tests and guinea-pig inoculations of milk sediment from all functioning quarters were

made on 49 cows whose history, sometime during their period of observation, showed blood agglutinins. In 38, or 77.5 per cent, of these, *Brucella abortus* was isolated from the udder one or more times. With the exception of 4 cows, the organism was recovered two or more times from the 34 udders, the milk of which was examined more than once. Two hundred and thirty-five such examinations were made between the years 1922 and 1927. In addition, 127 samples of milk from 32 cows which maintained a negative blood test during the entire period of study, were tested for *Br. abortus* by guinea-pig inoculation. In no case did a cow with *persistently* negative blood reactions show either the organism or agglutinins in the milk in the composite sample from the udder. However, in the group of 38 animals in whose udders *Br. abortus* was found one or more times, a significant fact developed in relation to blood agglutinins and udder infection: 8—or over 21 per cent—of the cows shed *Brucella abortus* from the udder either before agglutinins appeared in the blood or at a time when the blood agglutinins had receded to negative, or to positive at a dilution of only 1–50. Special attention is called to the blood and milk-test history of these cows.

The organism was recovered from the milk of cow 357 once when the blood was completely negative at 1–50 although this animal had a previous history of agglutinins at greater concentration. However, the titer had been practically negative in 1–100 for 18 months previously and her various tests show her practically free of agglutinins in the blood. Furthermore, *Brucella abortus* was recovered from a normal calving 7 months and 12 days before it was found in the milk.

Cow 334 shed the organism in the milk at four successive examinations at two-month intervals when the blood was twice negative in 1–50; once positive in 1–50 only; and once when partial in 1–100.

Brucella abortus was first present in the milk of cow 342 as long as 273 days, and again at 149 days, before the blood gave indications of agglutinins. The milk was positive for organisms on four succeeding tests over more than a year, though at these times the blood titer only once went over 1–200.

Cow 346 gave 7 positive milk tests for the organism over a period of practically two years. One of the positive tests occurred when the blood was negative in 1–50 and another when it was positive in 1–50 only.

Brucella abortus was recovered from the milk of cow 338, once 104 days and again 36 days before agglutinins appeared in the blood in a 1–50 dilution. After an interval, during which the blood became positive in 1–500 dilution, the organism was again found four consecutive times over a period of 240 days.

Cow 384 gave five positive milk cultures, one of which occurred when

only a partial reaction was present in 1–50 dilution of blood. The blood titer one month previous was partial in 1–100, and two months after this positive milk sample, positive in 1–50 only.

Cow 380 had a similar history, and *Brucella abortus* was isolated five consecutive times from the udder, at two of which times the blood titer was completely negative in 1–50 over a period of three months.

Brucella abortus was isolated from the udder of cow 349 in six consecutive tests. The first isolation was made after a two-year period of negative blood tests and 67 days before the next blood test showed her to have blood agglutinins.

Discussion of Blood Agglutinins in Relation to Udder Infection.—The data on this phase of the studies confirm the findings of many others, that the great majority of cows that harbor *Brucella abortus* in their udders show also sufficient agglutinins in the blood stream to be designated as positive reactors. However, the exceptions to the rule are of special interest from both the scientific and practical viewpoints.

The significance of the results of tests on the 8 cows that shed *Brucella abortus* while the blood agglutination was negative, or almost so, is apparent to anyone concerned with the control and eradication of Bang's disease. When approximately 20 per cent of udder-infected cows fail at certain periods to show sufficient agglutinins in the blood to be classed as reactors to the agglutination test, it is evident that this is at least one cause for the perpetuation of the disease in a herd and for the recurrence of reactors in certain herds where an eradication program is being carried out on the basis of the blood-agglutination test. However, the picture is not as dark as it might appear to be because the majority of animals of this type, in a herd that is frequently tested, would be identified and properly handled if intelligent supervision is operating. On the other hand, these data emphasize several points that must be kept in mind in the control of Bang's disease: (1) *Brucella abortus* in the udder does not necessarily stimulate the production of blood agglutinins; (2) frequent blood tests are necessary to identify the carriers; (3) cows showing agglutinins in the blood in low dilutions (1–50) may frequently be udder shedders.

Whey Agglutinins.—Unfortunately the data on whey agglutinins are of debatable significance in answer to the question of the relation of milk agglutinins to *Brucella abortus* in the udder because tests were made in all cases upon the milk equally mixed from all lactating quarters. Since the following data were accumulated, several investigators, as well as ourselves, have shown that only one or possibly two of the quarters may harbor *Br. abortus* and that the dilution of agglutinins, if present in these, by the addition of milk from the noninfected parts may

prevent a positive whey-agglutination test. While the method employed by us for isolation of the organism from the milk through sedimentation of the entire quantity drawn seems to have been reasonably satisfactory, the data on the related agglutinins merely confirm the findings of others as previously stated.

A total of 227 whey-agglutination tests of composite samples from all functioning quarters of 49 cows in this herd were made and each checked by guinea-pig inoculations. Ninety-three samples showed complete agglutination in the 1–50 dilution or higher and the organism was present in 66 tests, or in 70.9 per cent. One hundred and thirty-four were negative in the 1–50 dilution, and yet *Brucella abortus* was found in 43, or in 32 per cent, of the same samples.

When the cows are grouped to include those upon which two or more whey tests were made, and each one that showed agglutinins at any of the several examinations is designated as positive for agglutinins, *Brucella abortus* was isolated one or more times from 26, or from 83.8 per cent, of the 31 cows qualifying in this class. On the other hand, in 8 individuals that *never* showed agglutinins in 1–50 or higher in the mixed milk from the four quarters, 5, or 62 per cent, of them discharged the bacillus at some time during the period of testing.

Discussion of Data on Relation of Udder Infection and Udder Agglutinins.—Although one quarter only of an udder may contain *Brucella abortus,* most infected udders sooner or later have more than one quarter infected. Therefore it seems plausible that our data should have shown a higher degree of correlation between agglutinins and the presence of the organism in the composite sample of milk if this relation is of any practical value in identifying udder carriers. Our data do not indicate that the whey-agglutination test is of no value in identifying udder-shedders, but rather suggests its value as an aid in this procedure because a positive whey test in any dilution probably means udder infection. Conversely, however, the absence of agglutinins in a dilution of 1–50 was not positive proof that there were no *Br. abortus* organisms present in the same udder in this infected herd. Generally the agglutinins in the whey varied considerably in concentration from time to time and were usually lower than those in the blood.

CORRELATION BETWEEN BLOOD AGGLUTININS AND GENITAL INFECTION

In Positive Cows Calving Normally.—Normal pregnancies in 47 cows known as reactors were examined by culture and guinea-pig inoculation. *Brucella abortus* was recovered from 12 different animals, or 25.5 per

cent. Not all the normal calvings in these 12 animals were subjected to examination; in those examined more than once the germ was found twice in only one cow. Five of 6 pregnancies in one cow were examined and showed the germ only once. Four of the 12 had also a history of abortions with the organism present, and in 2 of these the abortions occurred subsequent to the normal pregnancy in which the parturition products were positive. Seven of the 12 cows had also a history of *Br. abortus* in the udder. The milk of the other 5 was not examined.

With respect to the titer of the blood serum at the time *Brucella abortus* was present in the uterus at the termination of a normal pregnancy, all except 2 such animals had a history for a considerable period before and after calving of blood reactions above 1–200, usually in 1–500. Of the exceptions, cow 357 had a positive blood reaction in 1–500 from July 28, 1922, to October 15, 1924, when the titer began to go down. Between March 15, 1925, and December 18, 1926, she gave 3 negative tests in 1–50. On the latter date the titer was up to a partial in 1–100. She calved 43 days later and her blood serum was partial in 1–50. Two tests, three and six months later, were completely negative in 1–50. In 11 tests over a period of three years after that time she showed 6 negative tests in 1–50 and 5 positive in 1–50. During these three years she had three normal calves, but *Br. abortus* was not recovered at any of the calvings. Cow 380 had a similar blood-titer history except that at a test 45 days before the calving at which the germ was recovered, she gave a partial in 1–500, and 27 days after calving was positive in 1–200. Before and after this period, during five years' testing, the titer varied from negative in 1–50 to partial in 1–100.

In Abortions by Positive Cows.—Attention has already been called to the rise in positive reactors and abortions in 1925, 1926, and 1928. The greatest number of abortions occurred in 1926, when 23 animals aborted. For the entire period of six years, 48, or 13.6 per cent of all pregnancies, terminated prematurely, of which 77 per cent were in positive cows, and 23 per cent in negative cows.

In the group of positive reactors there were 37 abortions. Thirty-six of these were given the routine tests for *Brucella abortus,* and the organism was isolated in 32, or in 88.8 per cent, of the examinations. Of the 37 aborting cows in this positive group, all except cow 347 were detected as positive reactors before the abortion occurred, by means of tests every three months. Cow 347 should be classed as a negative cow that aborted owing to *Br. abortus;* for she did not become positive until more than two months after the abortion. She is considered with the positive cows also, however, because of later temporary reactions. She had a negative agglutination history from April 2, 1923, to November 12,

1925. On August 27, 1925, she aborted, and *Br. abortus* was isolated by culture and by guinea-pig injections. The agglutination tests made 62 days before the abortion and 8 days after were both completely negative. On the following test two months later, the blood titer was only partial in 1–100, remained so during one year, and then became negative for another year.

In Abortions by Negative Cows.—An unusually large number of cows, negative to the agglutination test, aborted during the period of the investigations. There were 225 negative cows pregnant, of which 11, or slightly less than 5 per cent, aborted. All except one were given the routine laboratory examinations for *Brucella abortus,* with the recovery of the organism from two cows, 347 and 112. Only cows with completely negative agglutination histories are included in this group, with the exception of cow 347, previously discussed.

No particular effort was made to determine the cause of these abortions other than the tests for *Brucella abortus,* because they were well distributed throughout a six-year period; and since there was so much *Br. abortus* infection present in the herd, it was assumed that the majority of these cows would become positive reactors, an assumption that did not prove true with the particular group under discussion except possibly cow 347, referred to in the preceding group. Cow 112 was negative in 1–50 from June 22, 1922, to March 17, 1930, except for one test in September 16, 1927, when she was positive in 1–50 only. Six months and nineteen days after this test, with three negative ones in between, she aborted twin heifers. One of two guinea pigs injected with fetal stomach contents was positive for *Br. abortus.* She had had five previous calves, at normal term, and later gave birth to premature twins on two different occasions. Unfortunately, the last two premature births were not examined bacteriologically.

In Negative Cows Calving Normally.—In the study of negative cows calving normally, search was made for *Brucella abortus* in 134 of the normal pregnancies in 73 cows of the herd which had been consistenly negative to the agglutination test in dilution of 1–50. Among these, 4 cows, or 0.54 per cent, shed the organism at one normal calving.

Cow 138 was negative in 1–50 from December 18, 1925, to March 18, 1931, with the exception of a test on July 13, 1928, when there was recorded a partial reaction in the 1–50 and 1–100 tubes. No other plus sign appears in her record. Sixty-two days before the partial reactions noted, *Brucella abortus* was recovered from uterine swabs taken immediately after calving, by culture from one of two guinea pigs injected. The other guinea pig was negative.

Cow 322 was completely negative in all dilutions from 1–50 to 1–500 from April 2, 1923, to March 18, 1931. On March 3, 1924, she had retained placenta and parturient paresis, and two guinea pigs injected with uterine-swab suspension were positive for *Brucella abortus*. Two later calvings were negative.

Cow 140 was completely negative from February 19, 1926, to March 16, 1928. On September 16, 1927, she had her first calf. One guinea pig was positive by lesion and culture and the other had suspicious lesions, but the organism was not found in it.

Cow 16 was completely negative from July 8, 1922, to March 18, 1926. On October 10, 1925, she had her third calf. One of two guinea pigs inoculated with suspension from a uterine swab was positive for *Brucella abortus*. The other guinea pig was negative. Of two earlier calvings, one was negative and the other not examined.

Discussion of Data on Relation of Blood Agglutinins to Genital Infection.—The value of any biological test for diagnostic purposes lies essentially in its ability to consistently detect the animals that are carriers of infecting organisms or the products of their activities. It is only by the analysis of many data accumulated under varied conditions that such tests can be evaluated and their limitations circumscribed. In the data collected in this one herd under discussion, the agglutination test is shown to have a high degree of accuracy, yet it is subject to certain limitations, the knowledge of which is fundamental to practical interpretation and application.

In the group of positive cows that had some normal-term calves and some abortions, the data show that 20.3 per cent of the normal calvings were accompanied by the discharge of *Brucella abortus*, although it was found more than once in only 1 cow. But the more significant factors so far as the danger from these cows is concerned, were that two of the four abortions which had occurred in this group were subsequent to the positive normal calving, and the titer of 2 of the 12 cows had receded to 1–50 when they shed the organism at full-time calving. In this herd, therefore, 3.3 per cent of the cows that had been previously designated as positive reactors, and whose blood titer had decreased to and fluctuated between negative in 1–50 and positive in 1–100, were spreaders. These data illustrate the fallacy of retaining such animals in a herd in which an attempt is being made to eliminate Bang's disease, or of hoping that positive reactors may "get well" and be returned to the negative herd. Such cases may rarely occur, but this practice is certainly not to be recommended.

The ability of *Brucella abortus* to cause abortions and premature births was well shown in the calving records of this herd: 77 per cent of

the cows that were or became positive blood reactors, had prematurely terminated pregnancies, and in these *Br. abortus* was isolated from genital products of 88.8 per cent of the pregnancies examined. The blood agglutinins were closely correlated with the high percentage of culture findings; only one infected cow failed to indicate by her blood titer that *Br. abortus* was present in her body and was the probable cause of the abortion. However, among the 23 per cent of abortions that occurred in so-called negative cows, one certainly showed no signs of blood agglutinins in 1–50 over a long period of time and yet shed the organism from the genital tract. Another would ordinarily have been overlooked and the abortion laid to some cause other than *Br. abortus* if the blood titer had been taken as the only criterion. The data in this group, together with the finding of *Br. abortus* in four genital shedders among the group of always-negative cows that calved normally, point out certain limitations of the agglutination test that help to account for the difficulty that sometimes occurs in completely eradicating the disease from a herd in which a high percentage of infection originally existed. These negative cows that aborted or calved normally accompanied by the discharge of *Br. abortus,* were all tested by the agglutination test sufficiently long and frequently before and after calving to say positively that no agglutinin formation indicated the presence of the organism in the body. Since in three of the latter cases only one of two guinea pigs, injected from each cow, showed positive lesions and cultures, it may be assumed that the organisms were so few in number that a real infection was not established in the host.

The study of the agglutination-test reactions in this herd over a period of several years has shown the usual interesting and possibly disturbing variations in relation to known or unknown infection with *Brucella abortus.* The data, though not all included in this report, clearly divide the cows into three classes with reference to their blood titers: (1) those that remain persistently negative in all dilutions from 1–50 and above, (2) those that continue as definitely positive reactors from 1–200 and above, and (3) those that vary at different tests from negative in 1–50 to positive from that point upward. Perhaps a subdivision of the latter class would include those that show a definitely declining titer after a long period of high titers.

In group 1 are records of 36 cows, not including bulls, which have been tested regularly from five to eight years without a single positive sign in any of the many tests. Most of these cows have undoubtedly been exposed to *Brucella abortus,* for they have been in the herd while from 12 per cent to 36 per cent of the animals were reactors. All of them passed through the high peak of reactions and abortions in 1925 and

1926. Eleven of them have aborted, but *Br. abortus* was found in the uterus of only 1. Such data justify the conclusion that many cows have a high degree of natural resistance against ordinary herd exposure to Bang's disease.

Group 2 includes 14 animals that have given regularly high reactions in at least 1–500 for a period of from four to six years. Ten have aborted or discharged *Brucella abortus* at normal calvings. The agglutination test has a high rating in this group as a diagnostic test.

In the third class are 23 cows whose agglutination-test history over a period of from four to eight years is one of oscillating titers for lengthy periods, for the most part following high ones. This group is by no means free from the serious results of the infection because there are 10 abortions, 4 genital shedders at normal calvings, and infected udders to be found in it. It also contains most of the exceptions noted in the preceding pages regarding the correlation of blood and milk agglutinins to infection. The most practical point to consider with respect to these animals is whether or not they were free of the organisms after a period of negative blood tests following a history of infection. The records show that at least 5 of them were not, but were still carriers. More may have been.

The data from this herd are not extensive nor individual records complete in all cases on the subject of natural recovery from Bang's disease in the sense that the body no longer harbors the organisms, but they tend to the conclusion that it is rare in the average life of a cow. Some few have shown declining titers to negative tests after four or five years of positive reactions; but according to the data accumulated in these studies, great caution must be exercised in giving a clean bill of health to positive cows whose titers become low or negative over a considerable period of time. Some of these again show agglutinins and continue to be spreaders at irregular intervals.

FREQUENCY AND SEQUENCE OF ABORTIONS IN POSITIVE COWS

Under the conditions surrounding this herd, pregnant cows of all ages are assumed to have been equally exposed to the infection, although there may have been slightly less opportunity for first-pregnant heifers in that they were not in the milking strings. However, the space was limited and very general use was made of the same corrals for dry cows, heifers, and young bulls. So far as this herd is concerned, first-calf heifers showed less tendency to abort than did those in their second and third pregnancies.

BRUCELLA ABORTUS INFECTION IN A YOUNG BULL

In July, 1926, an Ayrshire bull, fifteen months old, in the herd but not yet in service, developed an orchitis in the left testicle (fig. 1). Abortion infection was suspected, and a blood sample taken agglutinated *Brucella abortus* antigen in 1–500 dilution. No higher dilutions were made. He had some contact with abortion-infected females from birth until eleven months of age. His dam had been a positive reactor at least since 1922 but had normal calves. As a calf the bull probably consumed mixed milk from the whole herd.

Fig. 1.—Ayrshire bull showing orchitis in left testicle due to *Brucella abortus*.

In order to ascertain whether or not *Brucella abortus* was being discharged through the urine, five samples of urine were collected during November and December. Approximately 1 liter was secured each time

Fig. 2.—*A*, Pathological testicle from Ayrshire bull shown in figure 1; *B*, normal testicle from same animal.

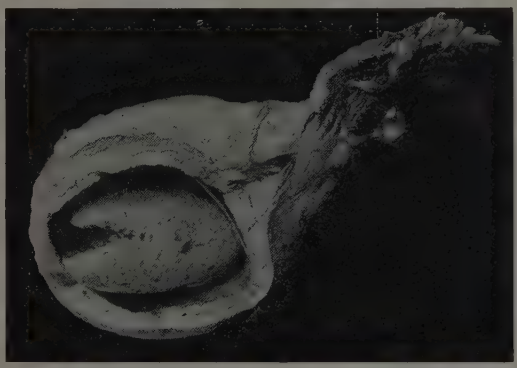

Fig. 3.—Appearance of opened pathological testicle from Ayrshire bull shown in figure 1.

and sedimented by centrifugation. Sterile saline was added to the sediment and 3 cc injected intraperitoneally into each of two guinea pigs. Several guinea pigs died in a few days from injections of the urine. The result of the injection into guinea pigs that lived to be autopsied and cultured show that the first sample, collected on November 15, 1926, con-

tained *Br. abortus* and that the other four samples were negative for the organism.

The bull was destroyed on February 11, 1927, and the following autopsy notes were made:

Infected testicle (figs. 2*A* and 3) measures 23 cm×13 cm when skin is removed. Normal testicle measures 15 cm×8 cm. The various tunics have undergone fibrosis to form a wall 1.5 cm thick. On section the wall shows numerous yellow foci 2 mm in diameter, which contain a caseous pus. The remains of the testicle float in approximately 500 cc of grey-yellow pus without odor, and is completely separated from its supporting structures. It resembles a testicle only in shape and size. The surface is of grey-yellow color and contains several crater-like erosions. The entire surface is roughened and has a granular appearance. Neither the vas deferentia nor epididymus can be observed within the fibrous sac. Outside the sac the spermatic vessels appear normal.

Cultures and guinea-pig inoculations were made from the following structures: prepuce, urethra, seminal vesicles, urine and mucosa from bladder, deep inguinal glands, infected testicle and normal testicle. At time of death the blood serum was positive up to 1–500. The results of the guinea-pig and culture inoculations showed the presence of *Brucella abortus* in the bladder and deep inguinal glands. Negative results were obtained from the other structures.

RESULTS OF SEPARATING THE POSITIVE AND NEGATIVE REACTORS

Failure to control the disease by sanitation and hygiene in connection with aborting cows, and the great increase in abortions that occurred in 1925 and 1926 led those in charge of the herd to divide the negative and positive cows so that the two groups occupied corrals and milking stalls on opposite sides of the milking barn. This type of separation was made on March 18, 1926. It was realized that this plan would not be wholly sufficient to prevent the spread of the disease, but other space was not then available. Table 1 shows the results of repeated agglutination tests and the separation of the negative and positive groups. Additional reactors were expected to be found since there were about 37 per cent reactors in the herd when the segregation was made. However, after a trial of eighteen months under the above plan, during which reactors were found in the so-called negative herd, all cows showing complete agglutination at 1–50 were removed to entirely new premises on the same ranch at a distance of ¼ mile. A special herdsman took charge of them and neither he nor any of the equipment came in contact with the negative group that was left on the old infected premises. No attempt was made to clean and disinfect the corrals to be occupied by the clean herd.

Since September, 1927, only 2 new reactors have been found, and none from the test of March, 1929, to the last test recorded in March, 1935.

Approximately 56 animals were removed from the herd as reactors to the agglutination test between September, 1927, and March, 1929. Fifteen of these became definitely sterile, 13 died or were destroyed for various disease conditions, and 18 were sold as reactors, and their history, from this time, is not available. During the entire time of segrega-

TABLE 1

RESULTS FROM REPEATED AGGLUTINATION TESTS AND SEGREGATION OF REACTORS

Date of test	Total tested	Reactors	Per cent	Date of test	Total tested	Reactors	Per cent
*March, 1926.....	115	43	37.4	September, 1929...	84	0	0.0
June, 1926.....	66	3	4.5	March, 1930.....	103	0	0.0
September, 1926..	66	3	4.5	September, 1930...	90	0	0.0
December, 1926...	75	1	1.3	March, 1931.....	91	0	0.0
March, 1927.....	71	1	1.4	September, 1931...	99	0	0.0
June, 1927.....	83	2	2.4	March, 1932.....	109	0	0.0
†September, 1927..	80	1	1.2	September, 1932...	117	0	0.0
December, 1927...	76	0	0.0	March, 1933.....	110	0	0.0
March, 1928.....	78	0	0.0	March, 1934.....	104	0	0.0
September, 1928..	81	1	1.2	October, 1934.....	97	0	0.0
March, 1929.....	96	1	1.0	March, 1935.....	94	0	0.0

* Following the March, 1926, test, the reactors were separated from the nonreactors by keeping the two groups in corrals on opposite sides of the milking barn when milked. The same caretakers handled both groups.

† On October 27, 1927, after the test of September, 1927, all of the reacting cows were moved to separate quarters about ¼ mile away and placed in charge of a special herdsman. On account of certain breeding experiments, it has been necessary to breed the reacting cows to negative bulls by bringing the former to neutral ground within the confines of the nonreacting group.

tion, the positive herd has been an economic liability because of sterility and an unusually high percentage of various disorders. All except 1 continued to give positive agglutination tests in a dilution of at least 1–500, until slaughter or death from disease. The one exception first reacted in 1922 and became negative in 1–50 in 1925, and continued to give negative reactions until September, 1930, when she again became positive in 1–200 for almost a year. Two of the original positive herd are still alive (May, 1935) but one is apparently sterile. Both have continued to be high reactors since 1925 and 1927 respectively, and both are still udder shedders.

SUMMARY AND CONCLUSIONS

Of the cows reacting positively to the blood-agglutination test, 77.5 per cent showed udder infection.

Cows in an infected herd may have *Brucella abortus* in their udders for a considerable period before agglutinins appear in the blood stream.

In 3 cases out of 38, the organism was present in the milk 8 months, 13 days; 5 months, 25 days; and 3 months, 14 days, respectively, before blood agglutinins were demonstrable.

The milk from udders harboring *Brucella abortus* frequently does not contain sufficient agglutinins to be detected by the usual whey-agglutination test in dilutions as low as 1–50 when a *composite* sample from all quarters is tested.

Udders of cows having a positive blood history may continue to carry *Brucella abortus* when the blood titer has declined to negative in a dilution of 1–50.

Cows with declining and low fluctuating blood titers may be spreaders at normal calvings when the titer is as low as negative in 1–50.

Of the cows reacting positively to the blood-agglutination and calving normally, 25.5 per cent expelled *Brucella abortus* at parturition.

Cows in an infected herd but with a consistently negative blood reaction in 1–50 over a long period of time, do occasionally (0.54 per cent) discharge *Brucella abortus* at a normal parturition. Likewise abortions in cows of this type were accompanied by the discharge of the organism with the fetus in 0.4 of 1 per cent of the abortions in this herd.

Recovery from Bang's disease is rare in the average life of a cow, and cows with a definite and long-standing positive blood history cannot safely be given a clean bill of health after a few negative tests.

Complete removal from the herd of all cows that show agglutinins in 1–50 was the only type of segregation that prevented the spread of Bang's disease in this herd. The data suggest that when this type of segregation fails, search should be made for nonreacting cows which are shedding *Brucella abortus* in the milk.

BRUCELLA ABORTUS SHEDDER CONDITIONS IN TWENTY COWS

B. S. HENRY, C. M. HARING, AND J. TRAUM

BRUCELLA ABORTUS SHEDDER CONDITIONS IN TWENTY COWS[1,2]

B. S. HENRY,[3] C. M. HARING,[4] AND J. TRAUM[5]

SINCE SCHROEDER AND COTTON,(1) and Smith and Fabyan(2) first demonstrated the presence of *Brucella abortus* in the milk of cows, there have been numerous reports of investigations carried on to determine the number of *Br. abortus* organisms excreted, the proportion of infected animals which eliminate the organism in their milk, the duration of this shedder condition, and its relation to blood-serum and whey tests. In most cases these reports have been based upon single tests of a large or small number of infected animals, and as would be expected the results have been exceedingly variable.

Fitch and Lubbehusen(3) found that 29.1 per cent of the cattle which were positive to the agglutination test were shedders of *Brucella abortus* in their milk, but that none of these organisms were found in the milk of animals whose blood-serum titers were less than 1–100 at the time of the test.

Results comparable with those of Fitch and Lubbehusen were obtained by Sheather,(4) who found that 34 per cent of the positive animals were shedders of *Brucella abortus* in their milk, but that 14 per cent of the samples of milk containing the organism gave negative results to the whey agglutination test.

In a group of cows with blood titers of 1–200 or over, Schroeder and Cotton(5) found that 83.3 per cent shed *Brucella abortus* in their milk. In a more recent study, Mitchell and Humphreys(6) reported 75 per cent of the animals in an infected herd to be shedders. However, if those animals in the herd whose blood titers were less than 1–100 were omitted, the percentage of reacting animals which were shedders would have been about 83. These last figures agree rather well with those obtained at this station, as shown later (page 551).

Pröscholdt(7) has stated that only 3 per cent of cows with blood-serum titers less than 1–100 eliminate *Brucella abortus* in the milk, but that cows with whey titers of 1–80 or higher almost certainly are shedders. He states that a whey titer of 1–10 to 1–40 is less certain evidence of infection.

[1] Received for publication July 9, 1935.
[2] This study was in part supported by a grant from the Bureau of Animal Industry, United States Department of Agriculture.
[3] Associate in Veterinary Science, resigned September 30, 1931.
[4] Professor of Veterinary Science and Veterinarian in the Experiment Station.
[5] Professor of Veterinary Science and Veterinarian in the Experiment Station.

Viridén[8] reports 50 per cent shedders among cows having a blood titer of 1–50, and 56 per cent among cows with a titer of 1–70.

Gwatkin[9] found 52 shedders among 102 cows showing blood-serum titers of 1–100 or greater. *Brucella abortus* was isolated from 3 out of 10 cows whose blood serums gave complete agglutination at the 1–50 dilution. One of these cows aborted and the agglutination titer never exceeded 1–50. What appeared to be a "vaccinal strain" of *Br. abortus* was isolated from the milk of a cow, the blood serum of which gave negative agglutination tests.

McNutt and Walsh[10] have described a cow that apparently was infected with *Brucella* for 21 months before she reacted, and a heifer which evidently carried infection for 13 months before reacting significantly.

Gill,[11] after inoculating a cow with *Brucella abortus* in the mammary vein, reported that the organism was shed from all 4 quarters for several months, and from 3 of the quarters for a complete lactation period, but no agglutinins appeared in the whey, although the blood titer became 1–800.

Thompson[12] found the inoculation of guinea pigs more efficient than direct cultures for detecting *Brucella abortus* in the milk. He considers that each quarter of the udder should be examined separately.

The observations of Hayes and Barger,[13] printed in this issue of Hilgardia, contribute important data on the relation between shedder conditions and the agglutination tests. They observed 3 cases in which shedder conditions existed for several months before a positive agglutinin titer developed in the blood.

METHODS

In this investigation the animals studied were in a so-called "infected group" which consisted of approximately 160 lactating animals, most of which were, or had been, reactors to the agglutination test, and had been segregated from a herd of approximately 800 animals. Some of the cows in this "infected group" had no history of *Brucella* infection. The entire herd was regularly tested for tuberculosis, and reactors removed.

After preparations for the experiment were completed, the first 23 animals which calved or aborted were taken for use in this study, so that no selection might be made which would influence the results. However, data from only 20 cows are presented in this paper; 3 of the 23 cows were removed from the herd because of reaction to tuberculin before representative data were obtained.

Blood and milk were taken from each animal within a few days after parturition, again 14 days after the first samples, and at approximately

monthly intervals thereafter throughout the lactation period. Except in the first part of the work, when the mixed milk from all quarters was used, the milk from each quarter was treated as a separate sample. After the udder had been thoroughly washed with germicidal soap and dried, and each teat immersed in an alcohol-iodine solution, about 150 cc of milk was drawn from each quarter and brought to the laboratory on ice.

A period of from 4 to 14 hours elapsed between the drawing of the samples and the inoculation of the guinea pigs. For these inoculations 70 to 80 cc of the milk was centrifuged at high speed for 20 minutes, the skimmed milk drawn off, and the cream and sediment thoroughly mixed. Two to 3 cc of this mixture was inoculated intraperitoneally into guinea pigs. The guinea pigs were killed 6 weeks later, and the presence or absence of *Brucella abortus* infection determined by spleen cultures, agglutination tests, and gross lesions.

Agglutination tests[6] by the tube method were made on all samples brought to the laboratory, using the serum of the blood and of the milk. To prepare the milk for the agglutination test, one drop of rennet was added to 10 cc of the decanted skimmed milk, which was then incubated at 37° C for 2 hours. The test was performed by adding 0.2 cc of the whey to 2.3 cc of antigen, and removing 1.0 cc of this mixture to a second tube containing 1.0 cc of antigen. This procedure was continued until 12 tubes had been used, giving dilutions of 1–12½, 1–25, 1–50, etc., up to a final dilution of 1–25,600. The initial dilution of 1–12½ was chosen for whey tests in order that subsequent dilutions would be comparable with those of the blood test, which was performed in the same manner except that 0.1 cc of serum was added to 2.4 cc of antigen, giving an initial dilution of 1–25.

For the purposes of this experiment, animals were classed as positive to the blood-serum agglutination test when there was a clumping of 50 per cent or more of the organisms in the 1–100 dilution tube. Animals were classed as negative when agglutination in the 1–25 tube was less than complete. Intermediate titers were classed as suspicious.

[6] The titers of the tests will be referred to in this paper as "blood titer" or "whey titer." Furthermore, for ease in stating the results of the tests, traces of agglutination in the terminal tube are disregarded, while 50 per cent or greater agglutination in a terminal tube is considered as complete agglutination in that tube. Therefore in a test where dilutions of 1–25, 1–50, 1–100, 1–200, etc. were used, a blood titer of $+ + \mp -$ is herein called 1–50, while $+ + \pm -$ is considered a titer of 1–100.

The antigen for both the blood and whey tests was prepared with *Brucella abortus* strain 80, isolated by Meyer and Fleischner, a strain now widely used in agglutination testing. It was suspended at a density of 3.5 cm on the Gates' opacimeter (2.5 on the McFarland). In all tests the antigen was sensitive but free from self-agglutinating organisms as shown by control tests with proved negative serums and with positive serums of known titer.

TABLE 1

MAXIMUM AND MINIMUM BLOOD AND WHEY TITERS AND BRUCELLA ISOLATIONS FROM MILK

Cow No.	Blood titer limits		Quarters*	Whey titer limits		Result of attempts to isolate Brucella abortus from milk by culture and guinea-pig inoculation																									
						Apr.		May		June		July			Aug.		Sept.			Oct.		Nov.			Dec.			Jan.		Feb.	Mar.	Apr.	May
	Max.	Min.		Max.	Min.	23	30	7	21	4	18	2	16	30	13	27	10	23	29	9	31	5	14	21	3	9	15	6	16	12	23	27	25
A-166	1–25600	1–400	1	1–800	1–200									+		+			+			+					+	+		+		
			2	1–1600	1–100									+		+			+			+					+	+		+		
			3	1–800	1–100									+		+			+			+					+	+		+		
			4	1–400	1–50									+		+			+			+					+	+		+		
A-880	1–25600	1–800	1	1–800	1–200									−		−		−			−	−						−		−			
			2	1–1600	1–400									+		+		+			+	+						+		+			
			3	1–1600	1–800									+		+		+			+	+						+		+			
			4	1–3200	1–200									+		+		+			+	+						+		+			
3419	1–25600	1–3200	1	1–400	1–50								−		−		−			−		−						−		−			
			2	1–3200	1–200								+		+		+			+		+						+		+			
			3	1–200	1–50								−		−		−			−		−						−		−			
			4	1–3200	1–200								+		+		+			+		+						+		+			
3191	1–12800	1–1600	1	1–400	1–50								−		−		−			−		−						−		−			
			2	1–800	1–100							+	+		+		+			+		+					+		+		+			
			3	1–800	1–100								+		+		+			+		+					+		+		+			
			4	1–1600	1–200								+		+		+			+		+					+		+		+			
6958	1–12800	1–800	1	1–1600	1–50								−		−		−			−		−			−			−		−		−	−
			2	1–800	1–50					−			−		−		−			−		−			−			−		−		−	+
			3	1–3200	1–100						+		+		+		+			+		−			+			+		+		+	
			4	1–400	1–50								−		−		+			+		+			−			−		−		−	
H-10	1–6400	1–200	1	1–200	1–12½							+	+	+		+						+			+			+					
			2	1–400	1–25	+		+		+		+	+	+		+		+				+			+			+					
			3	1–100	1–12½							+	+	+		+		+				+			+			+					
			4	1–50	1–12½							−	−	−		−		−				+			+			−					
512	1–6400	1–200	1	1–100	1–25									−		+		+				+					+	+		+			
			2	1–100	1–12½							+		−		+		+				+					+	+		+			
			3	1–200	1–25									o		+		+				+					+	+		o			
			4	1–400	1–25									+		+		+				+					+	+		+			
H-218	1–3200	1–200	1	1–50	1–12½								−		−		−			−		−						−	−	−			
			2	1–50	0								−		+		−			+		−					+		−		+			
			3	1–100	1–25			+	+		+		+		+		−			−		−					+		−		−			
			4	1–100	1–25								−		+		+			−		+					+		−		−			
2504	1–3200	1–400	1	1–3200	1–200									+		+			+			−					−	+		+			
			2	1–200	1–25	+	+	+		+		+		−		−			−			−					−	+		+			
			3	1–400	1–100									+		+			+			+					+	+		+			
			4	1–200	1–50									+		+			+			+					+	+		+			

7692	1-3200	1-400	1 2 3 4	1-400 1-200 1-200 1-100	1-100 1-50 1-50 1-50			+	+		+		++++		++++		++++			++++		++++				++++		++++					
23295	1-3200	1-50	1 2 3 4	1-100 1-100 1-100 1-100	0 25 0 0				−	+		+		−+−−		−+−−			−+−+			++−+					++−−	+−+−		++−+			
126	1-1600	1-100	1 2 3 4	1-200 1-25 1-100 1-25	1-12½ 0 25 0					+	+		+−+−		+−+−		+−+−			+−+−		+−+−				+−+−		+−−−		−−−−	−−−−		
4337	1-800	1-50	1 2 3 4	1-400 1-100 1-50 1-50	1-12½ 0 0 0								+−−−	+−−−		+−−−		++++				++−−				++−−		++−−		++−−	++−−		
A-828	1-400	1-50	1 2 3 4	1-12½ 1-100 1-200 1-100	0 0 0 1-12½					+	−		−−−+		−−−+		−−++			−−−+		−−−+			−−−+			−−−		−−−−	−− −	−−−−	− −
1009	1-100	1-50	1 2 3 4	1-25 1-50 1-50 1-50	0 0 1-12½ 0								−−−−	−−−−		−−−−			−−−−			−−−−					−−−−	−−−−		−−−−	−−−−	−−−−	
3325	1-200	1-25	1 2 3 4	1-12½ 1-12½ 1-12½ 1-12½	0 0 0 0			−	−		−			−−−−	−−−−		−−−−			−−−−		−−−−				−−−−		−−−−		−−−−			
A-632	1-25	0	1 2 3 4	0 0 0 0	0 0 0 0						−	−		−−−−		−−−−		−−−−				−−−−				−−−−		−−−−		−−−			
F-835	1-25	0	1 2 3 4	0 0 0 0	0 0 0 0			−	−		−		−−−−		−−−−		−−−−			?−−−		−−−−				−−−−		−−−−					
2262	1-25	0	1 2 3 4	0 0 0 0	0 0 0 0				−		−	−		−−−−		−−−−		−−−−				−−−−					−−−−	−− −		− −−			
2542	1-25	0	1 2 3 4	0 0 0 0	0 0 0 0			−	−		−		−−−−		−−−−		−−−−			−−−−													

* 1, Right front; 2, left front; 3, right rear; 4, left rear. Until July 16 mixed milk from the four quarters was tested.

c=contaminated.

RELATION OF BLOOD TITER TO SHEDDER CONDITION

While the elimination of *Brucella abortus* in the milk cannot be considered the sole criterion of active infection, any classification for the separation of positive and negative animals based on an agglutination test that does not remove the highest possible percentage of shedder cows from the negative group, is obviously inadequate. For this reason it is of interest to compare results of the periodic examination of milk with the corresponding blood titers.

From table 1 (p. 548–549) it will be seen that of the 20 animals considered in this investigation, 14 were proved to be shedders. In practically all of these shedder cows, the blood titers were well above 1–100 most of the time, the titer dropping as low as 1–50 in only 3 of the cows, and then only for a short time.

Brucella abortus was never isolated from the remaining 6 cows. The blood titers of these nonshedders were always below 1–100, except for cow 1009, which had 2 tests showing 1–100; and cow 3325, which on the first 2 tests had blood titers of 1–200 and 1–100 respectively. (See figs. 10, and 11, p. 566.)

Fig. 1.—Percentage of *Brucella* isolations from milk in repeated tests of 20 cows, grouped according to corresponding blood titers.

The total number of blood agglutination tests made in this study are grouped in figure 1 according to the number of tests falling into each titer division. Figure 1 shows that no isolations of *Brucella abortus* were made when the blood titer was below 1–50, but that when the titer was 1–50 the organism was obtained from the milk of one or more quarters

in 27.3 per cent of the 22 udder tests. When the blood titer was 1–100, 52.9 per cent of the tests yielded the organism. With a titer of 1–200, there were isolations in 86 per cent of the udder tests. At a blood titer of 1–400 or above, the organism was found in the milk of one or more quarters in from 95 to 100 per cent of the tests. In fact, of the 134 udder tests when the corresponding blood titers were 1–200 or over, *Br. abortus* was isolated in 96.3 per cent of the cases.

Although broad generalizations cannot be drawn from a group of 20 animals, it is obvious from the above that, considering only elimination of *Brucella abortus* in the milk, a standard which classifies animals as positive when the blood titer is 1–100 or over is by no means too severe.

In further support of the view that any cow which has a blood titer sufficiently high to cause any agglutination in a dilution of 1–100 or higher is potentially a shedder of *Brucella abortus* in her milk, the results of guinea-pig inoculations with milk from 210 cows in another project are presented. These animals all had a blood titer of 1–100 or higher. The mixed milk from all 4 quarters of each animal was used to inoculate the guinea pigs. The results were as follows:

10 cows had 4 tests; 10 (100 per cent) shed *Br. abortus* 1 or more times.
37 others had 3 tests; 34 (91.9 per cent) shed *Br. abortus* 1 or more times.
68 others had 2 tests; 55 (80.9 per cent) shed *Br. abortus* 1 or more times.
95 others had 1 test; 72 (75.8 per cent) shed *Br. abortus*.

Of the 210 cows, a total of 171 (81.4 per cent) were found to be shedders when tested from 1 to 4 times.

In summarizing the relation of blood titer to shedder condition, the data presented seem to indicate that (1) cows with a blood titer showing any agglutination at 1–100 or higher are actual or potential shedders of *Brucella abortus* in their milk; (2) cows with a titer of 1–50 may shed the organism at times.

RELATION OF WHEY TITER TO SHEDDER CONDITION

In recent years the use of whey rather than the blood serum for the agglutination test has been advocated from time to time. That this method has noteworthy advantages in ease of collection and in lessened disturbance of the animals from which the samples are being drawn, cannot be denied. However, there is still considerable disagreement concerning the delicacy of this test, and no agreement as to what constitutes a positive test.

Fitch and Lubbehusen[3] concluded that the titer of the whey was unsatisfactory as a means of determining the shedder condition, but on the other hand, Torrey[14] concluded that it is a comparatively simple mat-

ter to apply the rapid agglutination test to the whey from each quarter and thus determine the presence or absence of *Brucella abortus* infection.

From studies on 113 cattle, Gilman[15] concluded that milk to be used for agglutination work must be from individual quarters and not a composite sample from all 4 quarters. He found some correlation between the agglutination titer of the whey and the presence of *Brucella abortus* in the milk, and concluded that quarters showing agglutinins at a titer of 1–80 or higher are usually actively infected, but quarters with

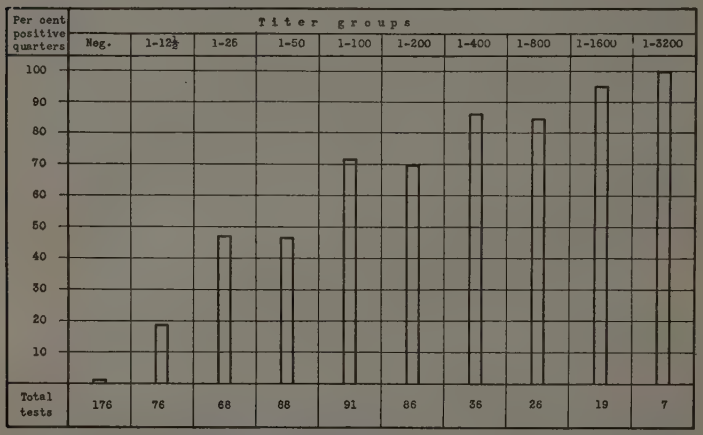

Fig. 2.—Percentage of *Brucella* isolations from milk of individual quarters of twenty cows repeatedly tested, grouped according to corresponding whey titers.

titers lower than 1–80 contain the organism only in rare instances. He found that of 108 cows which showed agglutinins at 1–80 or higher in one or more quarters, 78 per cent were infected.

Prouty[16] found 13 shedders in 18 cows that showed agglutination with 0.02 cc or less of blood serum by the rapid plate method (equivalent to 1–100 or higher by the tube method). All samples from quarters giving negative agglutination reactions with amounts of whey less than 0.08 cc (equivalent to 1–25 by the tube method) gave negative cultural findings for *Brucella abortus*.

Caldwell, Parker, and Medlar[17] are led to believe that the presence of agglutinins in whey is more reliable as an indication of udder infection than is their presence in the blood serum.

Beach and Humphrey[18] observed in a few cases that the colostrum whey of so-called "ceased reactor" cows showed agglutinin concentra-

tion of 1–400 or higher, but after the animals had been milked a few days the whey titer largely disappeared.

Figure 2 shows that isolations of *Brucella abortus* were made from 1 per cent[7] of the quarters showing no agglutinins in a whey dilution of 1–12½. The percentage of isolations increased in a relatively uniform line as the titer increased, until 100 per cent of the quarters with a whey titer of 1–3,200 were proved to be shedding the organism.[8]

Unlike the condition found in blood-titer groups, the percentage of shedders in the whey-titer groups did not abruptly ascend with the in-

TABLE 2

RELATION OF MAXIMUM MILK AGGLUTINATION TITER OF ANY QUARTER OF UDDER TO BRUCELLA ABORTUS SHEDDER CONDITION IN SAME UDDER

Titer of highest quarter in udder	Number of udders tested	Number of positive udders	Per cent of positive udders
Negative at 1–12½	35	0	0
1–12½	8	2	25.0
1–25	18	14	77.7
1–50	25	19	76.0
1–100	21	20	95.2
1–200	26	24	92.3
1–400	17	17	100.0
1–800	7	7	100.0
1–1600	9	9	100.0
1–3200	7	7	100.0
Total 1–25 or over	130	117	90.0

crease in titer above a certain point, and as may be seen from figure 2, it would be impossible in these tests to draw a line dividing the shedder animals from the nonshedders, based on the agglutination titer of the milk from individual quarters.

If, however, the whey titer from the quarter showing the highest concentration of agglutinins is taken as an index to the shedder condition in the entire udder, a rapid increase in the percentage of shedders occurs between the titers of 1–12½ and 1–25, as is shown in table 2. No *Brucella* organisms were found in any of the 35 udders tested when all quarters were negative. When the highest titer in any quarter of the udder was 1–12½, 25 per cent of the udders were found to be infected, while 77.7 per cent were infected when the highest titer of any quarter was 1–25.

[7] The 1 per cent represents 2 cows (fig. 7, p. 564, and fig. 9, p. 565), each of which had temporarily a negative whey titer in a quarter shedding *Brucella abortus*, but one of the other quarters in each cow showed a high titer at the same time and was also shedding the organism.

[8] 1–3,200 was the highest whey titer found in this study, although much higher titers have been encountered from time to time in connection with other projects. The highest titer found in whey tested at this laboratory was 1–204,800.

By an inspection of figures 3 to 9 (p. 561–565), the correlation between whey titer and shedder condition can be seen. In most cases the quarters shedding *Brucella abortus* from an udder had higher whey titers than the quarters not shedding the organism, as is also indicated in table 1. This is in agreement with the conclusion of Smith, Orcutt, and Little[19] that at least some of the agglutinins for *Br. abortus* are elaborated in the udder itself.

The mixed milk from all 4 quarters was tested on 16 occasions when the mixed whey showed no agglutinins in the 1–12½ dilution, and *Brucella abortus* was isolated 3 times. This indicates that mixed milk is not dependable for the detection of infected cows by the whey agglutination test, as a titer of 1–50 in a single quarter may easily be masked when the remaining quarters are negative or nearly so.

In summarizing the relation of milk titer to shedder condition, it is concluded from the above that a cow whose milk from any quarter shows, in repeated tests, a whey titer of 1–25 or over is probably eliminating *Brucella abortus* in her milk.

RELATION OF WHEY TITERS TO BLOOD TITERS

Although the blood titers of some of the animals fluctuated over a rather wide range, as may be seen from table 1, most of these cows maintained their status of positive or negative throughout the entire period. The fluctuation is far more marked in the whey titers, and changes from positive to negative occur in several cases. The most rapid change in the agglutination titer of the whey usually occurs within a short period after calving. A drop from a titer of 1–25,600 to 1–200 within 14 days, as shown in the case of cow 3191 (fig. 6, p. 563) has frequently been observed in infected animals when the initial test is made with milk drawn within a few hours after calving.

The blood titer was usually higher than the whey titer of any quarter. In some cases the whey titer is higher for a short period immediately after parturition (see figs. 3, 6, and 8, p. 561, 563, and 564, respectively). Twice a sample of milk from a single quarter showed a slightly higher titer than did the blood serum, but in both cases the next test showed a lower whey titer. Figure 9 (p. 565) shows one of these cases.

From the results of the repeated tests of the milk and blood in this group of animals, it is necessary to conclude that no cow would have been classed as negative by the result of the blood-serum agglutination test at any time when the whey titer was sufficiently high to cause the animal to be classed as positive according to our standard, with the possible exception of the short period immediately after calving.

CONSTANCY OF SHEDDER CONDITION

In the 14 infected cows studied, 44 quarters were found to be shedders of *Brucella abortus*. At the numerous times that the milk from each quarter was tested, 38 of these quarters were shedding the organism in from 33 to 100 per cent of the tests, while the organism was isolated only once from each of the other 6 quarters. The positive findings in these 6 quarters are difficult to explain on any grounds except errors in technic. Two of these (cows 6958 and 23295) were apparently due to switching of labels, as in each case an adjoining quarter, otherwise consistently positive, showed a negative test on the same date.

Of the 38 quarters repeatedly proved to be infected, 23 eliminated *Brucella abortus* at every test throughout the period of lactation. Milk from the remaining 15 quarters failed to infect guinea pigs on more or less irregular occasions, as is shown in table 1.

Further evidence that an infected udder may shed *Brucella abortus* continuously for months has been obtained in connection with an experiment carried on for other purposes. During this work the milk of a cow naturally infected with *Br. abortus* was inoculated into guinea pigs at an average of every 2 days for 78 days. All except 2 of these guinea pigs showed definite infection with *Br. abortus*. The failure to infect these 2 animals was probably due to some cause other than the absence of the organism from the milk. Although the milk from all 4 quarters of the udder of this cow was pooled for these inoculations, it was shown at the time the cow was autopsied that probably one quarter only was responsible for this continuous elimination of *Br. abortus*.

SPREAD OF INFECTION FROM QUARTER TO QUARTER

The ease with which cows are infected with *Brucella abortus* via the teat canals, and the permeable nature of the tissues separating the cisterns of the front and rear quarters on the same side of the udder, would suggest that infection established in one quarter would be apt to spread rather rapidly to the noninfected quarter of the same side of the udder. That such spread of infection did not occur to any extent is shown in table 1. In the 14 infected cows, there were 20 quarters which were negative at the first three tests, and which were therefore classed as negative quarters. At the termination of the study, 12 were found to have remained negative throughout the tests, 6 were positive on one occasion only, and only 2 had become definitely infected. Both of these had adjoining quarters on the same side of the udder which were always nega-

tive except for an isolation of *Brucella abortus* at one test only. The majority of quarters which remained negative during the entire period had adjoining quarters on the same side of the udder which were infected.

NUMBER OF BRUCELLA ABORTUS ORGANISMS EXCRETED IN MILK

While considerable data are now available concerning the number of *Brucella abortus* organisms found in the milk of infected cows at any one time, little has been done in determining the numerical regularity of the elimination. Unfortunately, the regular examinations of the whole milk from the separate quarters was not begun in this experiment until lactation had continued for a period of one or two months in most of the animals used.

The number of organisms in the milk was determined by culturing 0.1 cc of the sample in a petri dish containing solidified cooked-blood agar, to which had been added sufficient gentian violet to give a dye dilution of 1–208,000. Several investigators have reported the number of *Brucella abortus* per cc of milk to be very few. Similar results have been obtained in our work. The greatest number of organsims found in these studies was 3,020 per cc, and it was common to find only 100 to 200 or less per cc. Also, many samples which failed to show growth in cultures made from 0.1 cc of milk, contained sufficient number to cause growth when the sample was first concentrated by centrifuging.

A total of 186 counts of organisms were made, using whole milk from quarters which were shown by guinea-pig inoculations or cultures to be shedding *Brucella abortus* at the time. In 59 cases (31.7 per cent) infected quarters failed to show growth of the organism in the culture; 61 others (32.8 per cent) were found to contain from 10 to 100 *Br. abortus* organisms per cc; 40 others (21.5 per cent) contained from 101 to 500 per cc; 10 cultures (5.4 per cent) had between 501 and 1,000 per cc; 14 (7.5 per cent) showed between 1,001 and 2,000 per cc; the remaining 2 counts were 2,560 and 3,020 organisms per cc, respectively.

A comparison of the occurrence of colonies on cooked-blood agar plates with the blood and whey titers of the animals, shows that as the titers increase, *Brucella abortus* was isolated more frequently. For example, 5 counts were made of the organisms in infected milk from quarters with a whey titer of 1–25 while the blood titer was 1–200, and these samples showed 0, 0, 10, 40, and 350 per cc, respectively. The 5 counts made when the whey titer was 1–800 for each quarter and the blood titer 1–1,600, showed 20, 20, 50, 60, and 80 organisms per cc, respectively. Except that the number of organisms was greater in some cases, these

two examples are typical of the results in plating 186 samples from animals with whey titers ranging from 1–12½ to 1–3,200, and blood titers from 1–25 to 1–25,600. These examples also illustrate what was found to be true in general: namely, that when colonies did occur on the plates, the number of organisms per cc gave no evident index to the corresponding whey or blood titers.

In view of the constant nature of the shedder condition as shown in preceding sections, and the regularity in the number of organisms excreted by many animals, the 14 shedder cows studied may be considered as continual contributors of infection to the mixed milk of the herd.

STAGE OF LACTATION IN RELATION TO AGGLUTINATION TITERS

As previously stated, the agglutination titer of the whey was found to be high immediately after parturition, and usually dropped very rapidly in the first two weeks thereafter. After this sharp drop, the titer tended to remain relatively constant, but with occasional abrupt rises and falls until the end of the lactation period was approached. At that time a gradual increase in whey titer often occurred, which continued until the animal ceased to lactate.

The course followed by the agglutination titer of the blood was similar to that of the whey, except that no definite decline usually occurred after calving, nor was there any very marked rise in the blood titer at this time. Throughout the period the fluctuations were usually less than in the whey titers. Gradual increase in blood titers toward the close of lactation occurred in less than half of the animals.

The charts of the blood titers of the animals studied indicate that a diagnosis of shedder condition, based on several consecutive blood-serum agglutination tests, was possible and feasible at any time during the period of the tests.

OBSERVATIONS ON DISSOCIATION

In connection with a study of dissociation in the *Brucella* group, the *Br. abortus* strains isolated from the 14 infected cattle used in this project were systematically examined to determine whether or not dissociation played a part in the spread or maintenance of infection in brucelliasis of cattle.

For this purpose, 10 colonies (or all, if less than 10 were present) were picked at random from each positive culture during the period of the work. A total of 1,038 colonies of *Brucella abortus* thus picked were transferred and examined as to type. In addition, an equal, if not greater, number of colonies which appeared on the milk plates, and

which resembled but were not typical of *Br. abortus* colonies, were examined in the hope of detecting variant forms other than those obtained by the laboratory methods.

No *Brucella abortus* strains were obtained which were other than "S" in character and no atypical colony examined proved to be *Br. abortus*. From these data it seems probable that dissociation does not play an important rôle in the carrier condition in *Br. abortus* infection in cattle. This conclusion is in accord with the results obtained by Henry[20] with cultures from the milk of 114 cows in all stages of lactation.

SUMMARY

The blood and whey titers for *Brucella abortus* of 20 cows were studied at monthly intervals during a complete lactation period, and correlated with the shedder condition as determined by guinea-pig inoculations and cultures.

No milk sample was found to contain *Brucella abortus* when the blood titer of that cow at the time the milk was collected was below 1–50.

When the corresponding blood titer was 1–200 or over, *Brucella abortus* was isolated from the milk of one or more quarters in 96.3 per cent of the udder tests.

The percentage of shedders of *Brucella abortus* found among cows with blood titers of 1–100 or over, increased rapidly as successive tests of the cows were made over a period of time.

The diagnosis of shedder condition, based on several consecutive blood-serum agglutination tests, was possible and feasible in these 20 animals at any time throughout the period of the tests.

The agglutination titer of the whey is less dependable as an indicator of infection, and is more subject to fluctuations, than is the blood titer.

Brucella abortus was not recovered from any udder in which the milk from all individual quarters showed titers of less than 1–25.

In only 2 instances was *Brucella abortus* obtained from a quarter which had a negative whey titer, and in each case other quarters of the same udder had high titers and were excreting the organism.

Of 38 quarters proved to be infected with *Brucella abortus,* 23 eliminated the organism at every test throughout the period. The remaining 15 infected quarters failed to infect guinea pigs on more or less irregular occasions only.

The spread of infection from quarter to quarter, as indicated by the excretion of *Brucella abortus,* was very slow.

None but the "S" type of *Brucella abortus* were found in the milk of these naturally infected cows.

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APPENDIX

RECORDS OF TYPICAL INDIVIDUAL COWS

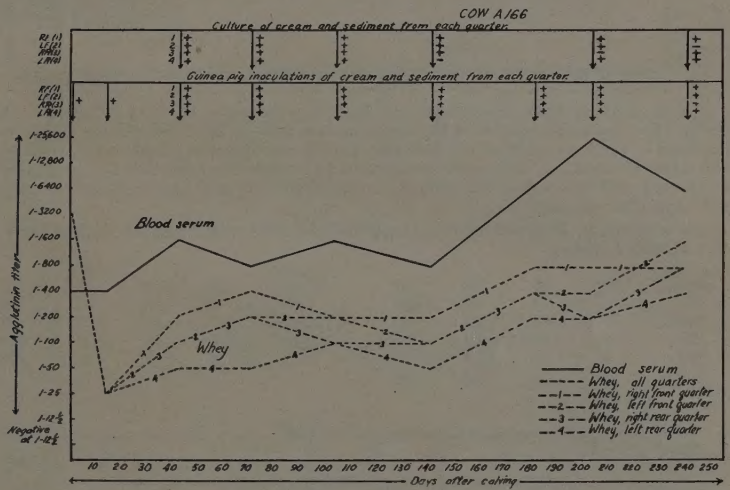

Fig. 3.—Cow A–166: calved June 18, 1930, dry February 20, 1931; eight-year-old Jersey; positive to the agglutination test for *Brucella abortus* for at least 18 months previous to the beginning of this study.

Note the sharp drop in whey titer on the fifteenth day after calving, which illustrates graphically the well-known phenomenon of antibody concentration in the colostrum. The gradual rise in titer at the end of the lactation period, as mentioned in the text (page 557), is shown here.

Counts of the number of *Brucella abortus* organisms per cc of milk from the four quarters of the udder, are as follows:

Days after calving	*RF*	*LF*	*RR*	*LR*	*Days after calving*	*RF*	*LF*	*RR*	*LR*
42	180	10	20	10	140	90	50	10	0
70	20	10	0	0	202 1,080		60	0	160
103	0	0	0	0	239	920	0	490	1,090

Tests made during the next lactation period indicated that the shedder condition persisted, and there was little change in the blood or whey titers.

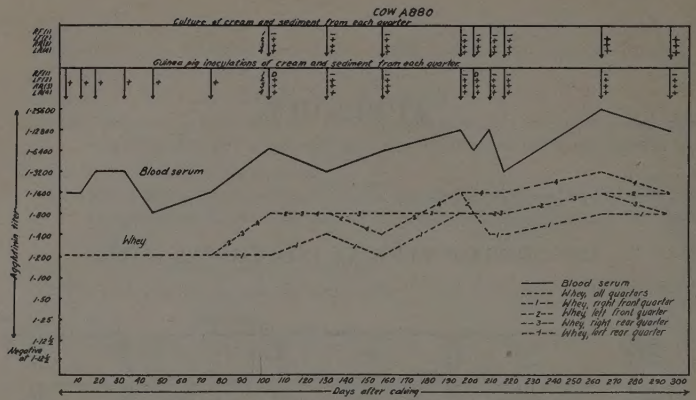

Fig. 4.—Cow A–880: calved April 20, 1930, dry February 12, 1931; nine-year-old Jersey; first became a reactor to the agglutination test for *Brucella abortus* 9 months previous to the beginning of the lactation period shown in the graph. At the end of 190 days of lactation, this cow's production fell below the minimum for profitable marketing, and the animal was brought to the laboratory and kept in lactation until 298 days after calving.

The numbers of *Brucella abortus* organisms per cc of milk from the various quarters were as follows:

Days after calving	*RF*	*LF*	*RR*	*LR*	*Days after calving*	*RF*	*LF*	*RR*	*LR*
101	0	60	30	220	208	0	50	—	70
129	0	80	20	50	215	0	60	20	50
156	0	50	10	70	264	0	30	20	230
194	0	40	30	40	298	0	30	20	110
200	0	50	0	20					

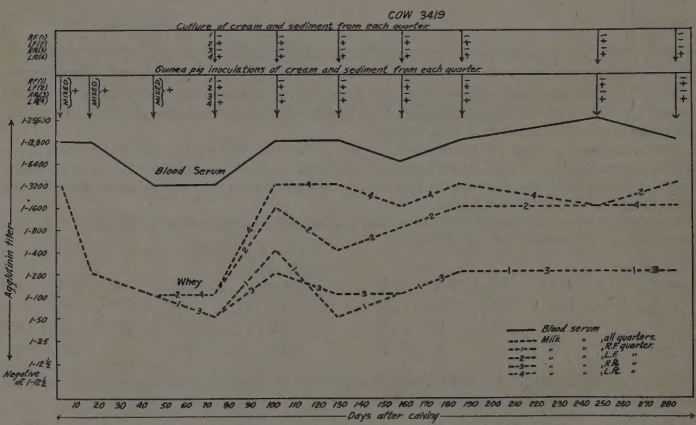

Fig. 5.—Cow 3419: calved May 5, 1930, dry February 27, 1931; nine-year-old Holstein; positive to the agglutination test for *Brucella abortus* for at least 18

(*Legend of fig. 5 continued on p. 563.*)

months previous to the first test shown on the graph. Previous calving was normal, with shedding condition during that lactation period.

Note the drop in the whey titer after calving, the relatively higher titer for the infected quarters, and the constancy of the shedder condition from the left half of the udder.

Numbers of *Brucella abortus* organisms per cc of milk from infected quarters during the lactation period shown in the graph were as follows:

Days after calving	*LF*	*LR*	*Days after calving*	*LF*	*LR*
100	90	360	184	90	1,200
128	630	1,360	246	1,580	1,070
157	80	970	283	0	170

Subsequently calved May 23, 1931, and three monthly tests of blood and milk after this calving showed little change from the previous lactation findings.

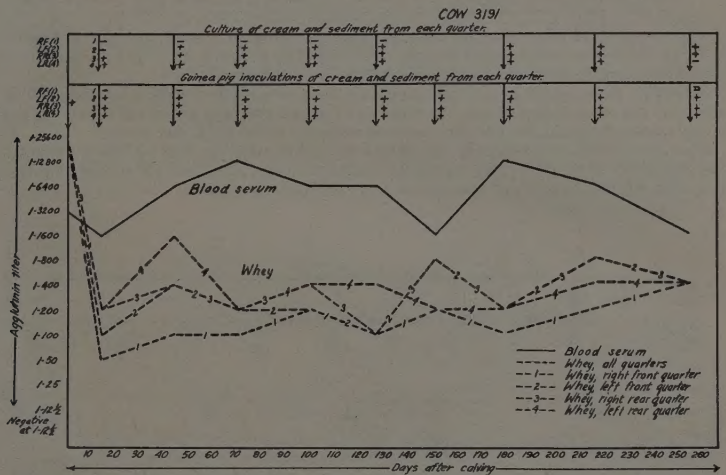

Fig. 6.—Cow 3191: calved July 2, 1930, dry April, 1931; eight-year-old Holstein; positive to the agglutination test for *Brucella abortus* for at least 15 months previous to tests shown on the graph.

Note the drop in whey titers soon after calving.

The numbers of *Brucella abortus* organisms per cc of milk from the infected quarters are as follows:

Days after calving	*LF*	*RR*	*LR*	*Days after calving*	*LF*	*RR*	*LR*
42	0	80	60	178	480	200	10
70	50	260	210	215	140	100	0
99	190	190	210	254	160	40	0
126	220	100	20				

A single test, made one month after the next calving, gave results comparable with those shown in the graph.

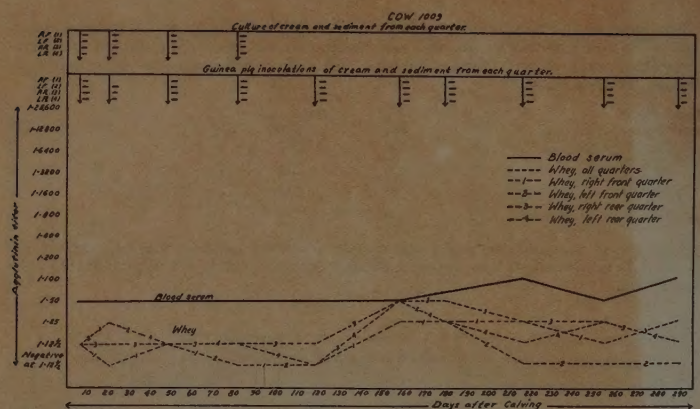

Fig. 10.—Cow 1009: calved July 10, 1930, dry May, 1931; seven-year-old Jersey. Agglutination tests for the preceding 16 months were all similar to those shown in the chart. The continuous low titers would seem to indicate that this animal had recovered from infection with *Brucella abortus*, and that the blood reaction noted was caused by residual agglutinins due to this infection.

The organisms were not recovered from the milk or cream of this animal at any time.

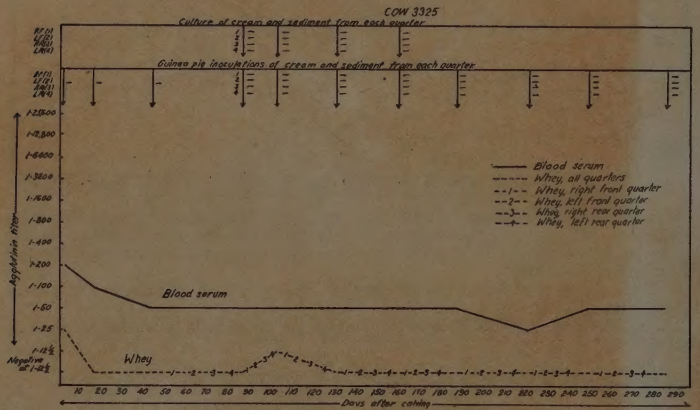

Fig. 11.—Cow 3325: calved May 5, 1930, dry February, 1931; eight-year-old Guernsey. Previous to this lactation, the agglutination tests for *Brucella abortus* were as follows: 16 and 14 months previous, negative; 12, 10, and 4 months previous, titers were 1–200; 23 days previous, titer was 1–50.

Brucella abortus was not recovered from the milk or cream during this lactation period, nor in three tests made after a subsequent parturition.